In order to test our dCas9 repression, we needed to have three different plasmids in our cells: 1) the dCas9 operon, 2) the part to be repressed (in our case, fluorescent reporter proteins), and 3) the gRNA designed specifically to repress the promoter controlling 2).

To transform in these three plasmids, we chemically transformed in the dCas9 operon, and used the cells from this transformation to create electrocompetent cells. We then electroporated these cells, simultaneously transforming in the plasmid containing the gene for our fluorescent protein and the plasmid containing the gRNA.

To make our electrocompetent cells:

1. Inoculate a single colony in 50ml LB broth with antibiotic
2. Incubate at 37°C in a shaking incubator overnight
3. Inoculate two 475ml LB flasks with 25ml overnight culture each
4. Incubate at 37°C in a shaking incubator until mid-log phase (approximately 1-2 hours)
5. Place flasks on ice and incubate for 30 minutes
6. Transfer culture to centrifuge bottles (250 ml). Centrifuge at 2,500 RPM for 15 minutes at 4°C.
7. Decant supernatant
8. Resuspend in 250ml ice-cold DI water
9. Centrifuge at 2,500 RPM for 20 minutes at 4°C
10. Decant supernatant
11. Resuspend in 125ml ice-cold DI water
12. Combine resuspensions into 250ml total volumes
13. Centrifuge at 2,500 RPM for 20 minutes at 4°C
14. Decant supernatant
15. Resuspend in 10ml ice-cold 10% glycerol in DI water
16. Centrifuge at 2,500 RPM for 20 minutes at 4°C
17. Decant supernatant and use a Pasteur pipette to remove remaining glycerol
18. Resuspend in 1ml chilled GYT (http://openwetware.org/wiki/GYT) by swirling (no vortexing or pipetting!)
19. In a cuvette, mix 990µl water and 10µl cell suspension.
20. Take the OD600 reading
21. Using the Agilent calculator (http://www.genomics.agilent.com/biocalculators/calcODBacterial.jsp) convert the

dCas9 Repression

OD600 measurement to cell concentration and dilute the original sample to $2.5e^{10}$ cells / ml with chilled GYT

22. Aliquot 50µL and freeze in liquid nitrogen
23. Store at -80°C until ready to use

To electroporate:

1. Add 20 µl GYT to the pre-made tube of electrocompetent cells
2. Add 0.5 µl of the plasmid to be repressed (~50ng) to the mixture of cells
3. Add 0.5 µl of the gRNA plasmid (~50ng) to the mixture of cells
4. Transfer the cells to a pre-chilled electroporation cuvettes
5. Wipe off the side of the cuvette to ensure it isn't wet, and tap the cuvette multiple times to eliminate bubbles
6. Put the cuvette into the electroporator, and shock at 1.6 kV
7. **Immediately**, add 1 mL of SOC
8. Grow for 1-2 hours at 37°C in a shaking incubator
9. Plate on LB plates selecting for appropriate antibiotic resistances (in our case, Amp, Kan, and Chlor)
10. Grow overnight at 37°C.

dCas9 Repression